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The Association of Yeasts with Certain Bark beetles

by

MOSHE SHIFRINE

B.S. (University of California) 1952

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

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Food Science

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Committee in Charge

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INTRODUCTION

The greatest loss of timber in California is caused by bark beetles. Since pines are California's main crop trees, the following four species of bark beetles which attack pines are of the utmost importance (Keen 1951).

1. Dendroctonus brevicomis Lec., western pine beetle, attacks ponderosa and coulter pines (Pinus ponderosa Laws., and P. coulteri D. Don.), (Whitesides 1951).
2. Dendroctonus monticolae Hopk., mountain pine beetle, attacks sugar, western white and ponderosa pines (P. lambertiana Dougl., P. monticolae D. Don., and P. ponderosa Laws.), (Evenden et al. 1943).
3. Dendroctonus jeffreyi Hopk., jeffrey pine beetle, attacks only jeffrey pines (P. jeffreyi Grev. et Balf.).
4. Species of Ips, pine engraver beetles, attack all pines.

The loss of pine timber resulting from bark beetle attacks in California, Washington and Oregon was estimated at approximately 15,545,000,000 board feet for the period 1931 to 1940 (see Figure I). For California alone, for the year 1951, losses were reported at 400,000,000 board feet. The estimated loss for 1952 appears even greater than that for the 1951 period (Callaham 1952).

Caird (1934) reported on the mode of attack by bark beetles and its effects on the tree. The beetles bore through the bark of the attacked tree and tunnel into the phloem on which they feed. The entrance holes and the adult and larval galleries expose the wood to the air, thereby disrupting the water conducting system. The outer rings of the tree first lose the capacity to conduct solutions. Next the inner rings

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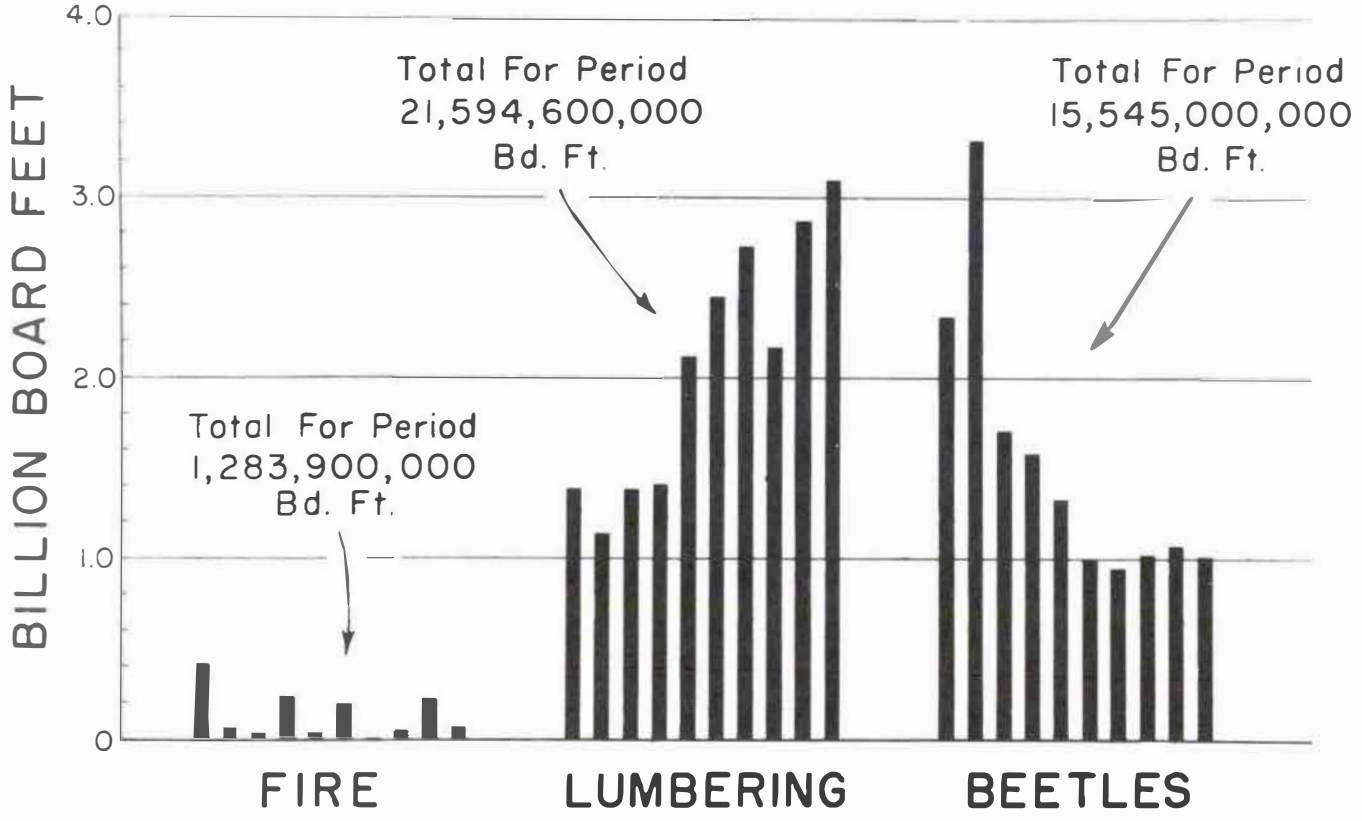
COMPARISON OF TIMBER DRAIN

Pine Region – California, Oregon and Washington

1931 – 1940



fail in their ability to transfer solutions. This cuts off the supply of water to the needles which deplete the top of the tree of water, and the tree dies of desiccation. Failure of the outer rings to conduct solutions causes drying of the wood which permits penetration into it by Ceratostomella pini Münch. This blue-staining fungus penetrates deeply into the wood during the early stages of attack, filling the xylem tissues with a mass of mycelium. The association of blue-stain fungi with bark beetles has been observed for a long period of time. However, the significance of bark beetles as vectors of the fungus was not discovered until Craighead (1928) questioned the ability of the beetles alone to kill trees in a short period. Experiments conducted by Nelson and Beal (1929) showed that blue-stain fungi, together with the wounding which accompanies inoculation, may kill pines in a comparatively short time. Caird (1934) and Bramble and Holst (1935) showed experimentally that C. pini alone is capable of killing trees when used for inoculation, provided that the inoculation points encircle the trunk of the trees.

With all the species of bark beetles the first evidence of attack on living trees is the presence of pitch tubes or boring dust on the trunks. Later, the fading yellowish, or reddish color of the foliage is conspicuous evidence of the bark beetles' destructive work. All of the studies on attacks by Dendroctonus beetles have demonstrated their ability to attack certain apparently healthy trees and kill them, wherever the individuals of a species occur in sufficient numbers to overcome the resistance of the tree (Hopkins 1909).

A few investigators have reported on the association of yeasts with bark beetles. However, information on the nature of this association and types of yeasts involved are incomplete (Person 1929, Struble 1930, Crossman 1930, and Holst 1936).

There are two theories of tree selection by western bark beetles, in both of which yeasts play a predominate role (Person 1931, Callaham 1952). The attack of a tree by bark beetles includes two stages. First there is the stage of initial attack by a relatively small number of beetles. One theory maintains that this initial stage of attraction is the result of some volatile substance being produced by a susceptible tree. However, there is no experimental evidence to support this theory. According to the second theory the first beetles attack trees randomly, without preference. The second stage is common in both theories of tree selection. In this stage, 3 to 10 days after the initial attack, a much more definite attraction is evident. This seems to be the result of phloem fermentation by the yeasts brought in with the first beetles. The end products of the fermentation attract other beetles en masse. That fermented phloem attracts beetles has been shown experimentally by Gordon (1933). The result of the mass attack is the death of the attacked tree in about two to three months. The present-day hypothesis of host susceptibility is based first on oleo-resin production as a means of host resistance and second on the ability of yeasts to establish themselves in the phloem after being introduced into the tree by the beetles.

Because of the very limited knowledge on the nature of the yeasts associated with various bark beetles, it was decided to isolate and identify a number of these yeasts and study some of their properties.

In some cases, yeasts were isolated from all stages of the life cycle of the beetles, with the exception of the eggs. It was hoped that a little knowledge of the yeasts associated with the beetles, might constitute a first step in a better understanding of the biological significance of the yeasts in the relation between the beetles and the trees which they attack.

REVIEW OF THE LITERATURE

The association of microorganisms with insects has been known since the sixteenth century (Steinhaus 1946). However, the first observer to record the presence of living microorganisms in insects was Raimbert (1869), who obtained anthrax bacilli from flies exposed to the cadavers of infected animals. Since then a voluminous literature has appeared dealing with various aspects of this association. The nutritional study of insects with special reference to microorganisms and their substrata was covered in detail by Baumberger (1919). In his paper Baumberger grouped the feeding habits of insects into the following three classes:

1. Ingestion of microorganisms with the substratum, e.g., Drosophila, Musca and worker termites.
2. Feeding directly on microorganisms, e.g., mites, tree crickets, and many adult diptera.
3. Preparation of a medium for microorganisms, e.g., leaf cutting ants, termites, and ambrosia beetles.

No work had been done, at that time, on the western bark beetles and

consequently they were not classified in any of the three classes. Uvarov (1928) summarized the literature on the nutrition and metabolism of insects, emphasizing the great lack of accurate and specific information on the subject. Wingard (1925), Fawcett (1929), Wallace (1932), and Leach (1940), discussed the transmission of plant pathogenic yeasts and other microorganisms by insects. Steinhaus (1946) in his book "Insect Microbiology" completely reviewed the literature dealing with yeast-bark beetle association. Mrak and Phaff (1948) thoroughly reviewed the ecology of yeasts in insects, including the association of yeasts and bark beetles. Schneider-Orelli (1913) stated that yeasts were always present in the tunnels of ambrosia beetles. An extensive study on this subject was done by Shirley Webb (1945). It has long been recognized that ambrosia beetles cultivate in their galleries a fungus upon which they, and their larvae, subsequently feed. Webb studied the growth characteristics, appearance in the tunnels, and the relationship of ambrosia beetles to fungi. Studying the association of yeasts with these beetles she isolated species of Endomycopsis that were constantly present in the tunnels of the larvae and adults. Verral (1943) isolated two species of yeasts, commonly associated with southern species of ambrosia beetles, which he named Endomyces bispora and Monilia brunnea. Verral thought that these fungi were used as food by the ambrosia beetles with which they were associated and that they probably caused restricted black and brown stains adjacent to the beetles' tunnels.

Beck (1922) described a new species of yeast Endomyces bisporus, which she found associated with the bark beetle Ips typographus attacking spruce and fir trees in Austria. In 1939 Siemaszko again isolated

this yeast species from the same species and in the same species of trees, but this time in Poland. Grossman (1930) in her work on the association of bark beetles and blue-stain fungi, regularly isolated yeasts from the galleries of Ips typographus. She also found large numbers of yeasts in the intestinal tract of the larvae and viable yeasts in their fresh excreta. She postulated the possibility that the beetles used the fungus enzymes for the digestion of food. She reported three nonfermenting types of yeasts, which were described as follows: 1) A budding yeast having hat shaped ascospores, 2) a yeast similar to the first type but forming mycelium, and 3) a type forming mycelium but no ascospores. One of the yeast strains isolated was identified as Endomyces bisporus Beek. As a result of her investigations of the symbiosis problem Grossman concluded the following:

1. The yeasts are transferred from tree to tree by the mother beetle. The infection of the larvae with yeast takes place by their creeping and feeding on the bark.
2. The yeasts are not absolutely necessary for the development of the insects, they may be simply regarded as universally present.

However, it appears to the author that the second conclusion is not warranted from the experiments conducted by Grossman. Definite and explicit evidence has to be furnished before the decision can be made that yeasts should be regarded as merely universally present.

Leach, Orr and Christensen (1934) found characteristic yeasts constantly associated with bark beetles and blue-staining fungi in felled Norway pine timber.

In 1936, Holst isolated a yeast that was found to be generally associated with bark beetles belonging to the genera Ips and Dendroctonus.

The yeast was subsequently named Zygossacharomyces pini nov. sp. (later renamed Saccharomyces pasteri by Lodder and van Rij, 1952). This yeast may have been identical with the first type of Grossman. Studying the association of bark beetles and blue-staining fungi, Rumbold (1941) observed the presence of Z. pini and several species of Candida in two species of Dendroctonus. She also noted that, in making cultures from the tunnels around the beetle galleries, yeasts were always the first organisms to appear, the blue-staining fungi only developing later. The different species of yeasts grew on sterilized pine on which the blue-stain fungus did not grow. The yeasts appeared to have a stimulating effect on the staining fungus, allowing it to grow more vigorously on agar.

not in lit. cited

Holst (1937) grew larvae of Ips species aseptically, and presented evidence that neither the yeasts nor the blue-staining fungi, so generally found in association with Ips species, were essential for the development of the adult beetle from the egg. The author believes that the few experimental results described in the paper by Holst do not warrant the aforementioned conclusion.

Pekle and Satava (1950) stated that pure cultures of symbionts from larvae of Ips species were able to fix atmospheric nitrogen, making it available to the insects. Claims that yeasts can fix atmospheric nitrogen have occasionally appeared in the literature, but no definite confirmation by other workers has ever been forwarded to substantiate these claims. Pekle and Satava do not describe their experimental procedures, therefore it is impossible to be sure that their microorganisms can fix nitrogen. In this connection Baumberger's (1919) theory states

that insects inhabiting fermented and decaying substrata of low protein content usually feed upon the microorganisms present and thus benefit by the power of the fungi to transform inorganic nitrogen compounds into complex organic nitrogen.

Thus far we have seen that the nature of the ecological relationships between the bark beetles and the yeasts associated with them is inadequately known. Much of this has been due to the use of poor systems of yeast taxonomy. Since many advances have been made in this field during the last twenty years, it was felt that a more extensive study of the yeasts associated with various bark beetles would yield fruitful results.

The ultimate purpose of the present project undertaken at the request of the Division of Forest Insect Investigations, U. S. Department of Agriculture, Bureau of Entomology and Plant Quarantine, is to obtain information on the following points:

1. Is the composition of the yeast flora, associated with each species of bark beetle, relatively constant with respect to:
   a. Geographical distribution?
   b. Different stages of the life cycle?
   c. Different host species which the beetles might attack?
2. Does each yeast flora produce an attrahent specific only for its associated bark beetle species, or rather is it attractive to many species?
3. Can the attrahents be ultimately produced and used in direct control methods?
4. Is the composition of the yeast flora at all critical in determining

the success or failure of brood development in different host species which a given insect species might attack?

The answers to these questions are indispensable to an understanding of host selection, host resistance, attraction, and bark-beetle biology.

The initial objective of the particular project, covered in this thesis, was to isolate and identify the yeasts associated with each of the metamorphic stages of some of the more important bark beetles removed from different pines.

EXPERIMENTAL METHODS

1. Collection of bark beetles

The first beetles were collected, in the summer of 1952, from El Dorado and Lassen counties, California, and from Crystal Bay, Lake Tahoe, Nevada. The sources of the respective beetles are given in Table II. The majority of the collecting was done at Blacks Mountain, Lassen County, California, to avoid introducing possible geographical variables of yeasts into the investigations. This might have complicated the study of the interrelation between yeasts, bark beetles and pines. Brood material of each insect was brought into the laboratory and stored until the bark beetles were removed and dissected. In August 1952, a second trip was made to Blacks Mountain Experimental Forest. At that time beetles were removed from attacked trees and dissected immediately. The advisability of avoiding delay between removal of the beetles from the tree and subsequent dissection was suggested by observations made,

during the spring of 1952 (Phaff and Shifrine), with Dendroctonus valens Hopk. In this case the digestion of the intestinal flora took place within a few days, when the beetles were kept in storage outside the bark or phloem prior to dissection. Further investigations confirmed that the intestinal flora of the beetle is digested on storage especially at elevated temperatures. If dissection cannot be done immediately, the beetles should be stored temporarily under refrigeration. The above observations are quite in line with those of Shihata and Mrak (1952) who studied digestion rates in Drosophila, and Miller (1951) working with Carpophilus.

2. Dissection of beetles

The purpose of dissection was to isolate only those yeasts present in the intestinal tract. In order to avoid isolation of yeasts adhering to the insect body, due to accidental contact with extraneous matter, preliminary experiments were conducted to perfect a method of sterilizing the surface of the beetle. At first a 0.1% solution of $HgCl_2$ was used. The beetle was submerged for one minute, then washed in sterile water. On subsequent smearing of the beetle on a wort agar plate, without dissection, yeast colonies appeared. Therefore, the strength of the $HgCl_2$ was increased to 0.5%. Further experiments showed that with 0.5% solutions the beetle has to be dipped for five minutes to insure complete surface sterilization. This method proved to be satisfactory for the adult beetle, larva and pupa.

The aforementioned procedure was not tried with eggs of bark beetles. A method for the sterilization of eggs was reported in the literature by Holst (1937). With the aid of a small camel's hair brush

Holst washed eggs of Ips for twenty seconds in 95% ethanol. No use of $HgCl_2$ solution was mentioned.

After treatment with $HgCl_2$ the beetles were transferred to a test tube containing sterile water, and then washed with sterile water.

After sterilization and washing the beetle was mounted dorsal side up on a sterile paraffine block, by melting a small area of paraffine and allowing it to harden after placing the beetle in the melted wax. A scalpel made of a triangular piece of razor blade attached to a small glass tube was used to dissect the beetle. This procedure was carried out under a wide field microscope (ca 20X). After mounting the beetle on the paraffine block, both the elytra and wings were removed with the aid of very tiny needles. The head then was severed and a longitudinal cut was made from the prothorax through the metathorax and abdomen, without severing the gut. The cut was then widened with the aid of two needles, and the alimentary canal was pulled out and placed on a wort agar plate. The intestinal tract was pulled apart with needles and the content was streaked with a loopful of sterile water over the surface of the plate. The above procedure is described by Miller (1951) in his work on Carpophilus. A pupa or larva was dissected by placing it on a sterile slide, under a wide field microscope, and pulling it apart with two needles. The content then was streaked with a loop over the surface of a wort agar plate.

3. Isolation of yeasts

First to appear on the streaked plate were yeast and bacterial colonies. The number of yeast colonies to appear on the plate varied from one to over three hundred. Most commonly between 50 and 100 yeast

colonies appeared on a plate. About a week later mold growth started on most of the plates. The yeast colonies were inspected both macroscopically and microscopically. An established yeast colony was removed with a sterile needle, suspended in sterile water and restreaked on another wort agar plate. This procedure was done twice. When the colonies on the first plate were not homogeneous in appearance, or when morphological differences were noticed on microscopic observation, all the different colonies were isolated. Usually only one type of yeast was found on each plate (Table I). Occasionally two yeasts were isolated from one plate, and rarely three. No more than three different yeasts were ever found on one plate.

4. Identification of yeasts

For the determination of the morphological and physiological characteristics of yeasts, methods used by the Dutch workers, Stelling-Dekker (1931), Lodder (1934), Diddens and Lodder (1942) and Lodder and van Rij (1952), were followed with few exceptions.

The ability to ferment different sugars is one of the main characters for yeast classification. Therefore, Durham rather than Einhorn tubes were employed for fermentation tests because of their greater sensitivity (Bouthilet et al. 1949). To avoid erroneous results of fermentation a medium of 0.5% yeast autolysate (Difco) was used, rather than yeast extract as prepared by the Dutch workers. The latter extract may contain measurable amounts of fermentable trehalose (Bouthilet et al. 1949). The ability to ferment glucose, sucrose, galactose, maltose, and lactose was determined for all yeasts. In cases in which the yeast was able to ferment sucrose, the fermentation of raffinose was determined

TABLE I

Number of beetles dissected in the different stages of their life cycle and the corresponding number of yeasts isolated

Stage in life cycle	No. of individuals dissected	No. of individuals yielding no growth	No. of yeasts isolated
Adults in flight	4	1	3
Adults before emergence	98	22	97
Callow-Adults	15	0	16
Pupae	17	2	17
Larvae	22	3	18
Total	156	27	151

by the method of Wickerham (1943), rather than in van Iterson-Kluyver fermentometers as used by Lodder and van Rij (1952).

The ability to assimilate glucose, galactose, maltose, sucrose, and lactose was determined for all yeasts. In some cases the ability to assimilate alpha-methylglucoside, erythritol, rhamnose, raffinose, and trehalose also was determined to permit identification by the classification system of Wickerham (1951).

Sporulation media employed included: wort agar, vegetable-extract-agar plus 1% dried yeast, vegetable extract agar, carrot and potato wedges, Gorodkowa's agar, Acetate agar (Powell 1952), and Jeffrey pine phloem strips.*

The occurrence of carotinoid pigments was determined by the method described by Mrak, Phaff and Mackinney (1949).

The ability to utilize ethanol as the sole source of carbon, was determined in a synthetic medium containing Wickerham's nitrogen base and 3% ethanol.

Carbon assimilation tests were studied, in addition to the auxonographic technique, by the replica plating technique described by Lederberg and Lederberg (1952). Washed agar with 0.5% of the carbon source studied was used for replication. The development of colonies was read after 3 and 7 days and compared to a blank plate without a carbon source and one containing glucose.

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*A section of a young pine (Pinus jeffreyi) was brought to the laboratory immediately after it was felled. The thin bark was separated from the wood, and the phloem was cut into strips which were placed in a metal container, and stored for 24 hours at -20 C. The strips then were put into large test tubes (25 X 250 mm) and autoclaved at 15 lbs pressure for one minute. The strips of phloem were inoculated when cool.

In general, the taxonomic treatment used was in accordance with that used by Lodder and van Rij (1952).

INVESTIGATIONS

1. Isolation of yeasts from Dendroctonus and Ips bark beetles

A total of 156 individuals, belonging to the genera Dendroctonus and Ips, were dissected at different metamorphic stages, yielding 151 yeast cultures. Of the 156 individuals dissected 27 yielded no microbial growth. This, probably, was the result both of the dried condition of the intestinal tract in some insects, and possibly of faulty technique. In a few cases the plate, smeared with the intestinal tract of a beetle, contained only bacterial colonies, which were not isolated. In no case did mold growth interfere with the isolation of yeast colonies. Table I shows the number of individuals dissected, their metamorphic stage, and the corresponding number of yeasts isolated.

The first beetles dissected were collected from logs brought to the laboratory from field infestations. Due to the variations in brood development in this material, it was not possible to secure an adequate sample of each metamorphic stage of each insect species from its various hosts. However, an adequate sample of new adults was obtained from this material to determine the inter- and intra-generic variations in the yeast floras associated with the adult beetles. It also was felt that there might be significant variations in the yeast flora associated with the successive metamorphic stages in the life cycle of any one insect. On this basis additional collections and dissections were made in the

field to obtain a complete sample of the metamorphic stages of Dendroctonus monticolae Hopk., and Ips oregoni Eichh.

The yeasts isolated represent seven genera and thirteen species: Hansenula capsulata Wickerham, H. sylvicola Wickerham, Saccharomyces pastori (Guilliermond) Lodder et van Rij, S. bisporus (Naganishi) Lodder et van Rij, Pichia haplophila nov. sp., Candida sylvicola nov. sp., C. parapsilosis (Ashf.) Langeron et Talice, C. parapsilosis var lactosa nov. var., C. rugosa (Anderson) Middens et Lodder, C. mycoderma (Reess) Lodder et van Rij, Torulopsis pinus Lodder et van Rij, Torulopsis nitratophila nov. sp., Rhodotorula crocea nov. sp., Cryptococcus diffluens (Zach) Lodder et van Rij. The majority of the 151 isolates fall into four species, H. capsulata (36 cultures), C. sylvicola (69), C. parapsilosis (16), and S. pastori (13). The number of isolates belonging to each of these groups is shown in Figure II. A taxonomic discussion of the various species will appear later in the thesis.

The species of beetles dissected, the taxonomic designation of the yeasts isolated, and the species of pine from which the beetles were collected, is shown in Table II. The beetles dissected represent six different species of bark beetles: Dendroctonus jeffreyi Hopk., D. monticolae Hopk., D. valens Hopk., Ips oregoni Eichh., I. confusus Lec., and I. emarginatus Lec. These were collected from five different species of pine, Pinus jeffreyi Grev. et Balf., P. monticola D. Don., P. ponderosa Laws., P. contorta var latifolia Engelm., and P. lambertiana Dougl.

Twenty-four yeasts were isolated from all the metamorphic stages (except the eggs), in the life cycle D. monticolae collected from P. monticola. The number and species of yeasts isolated from each of the

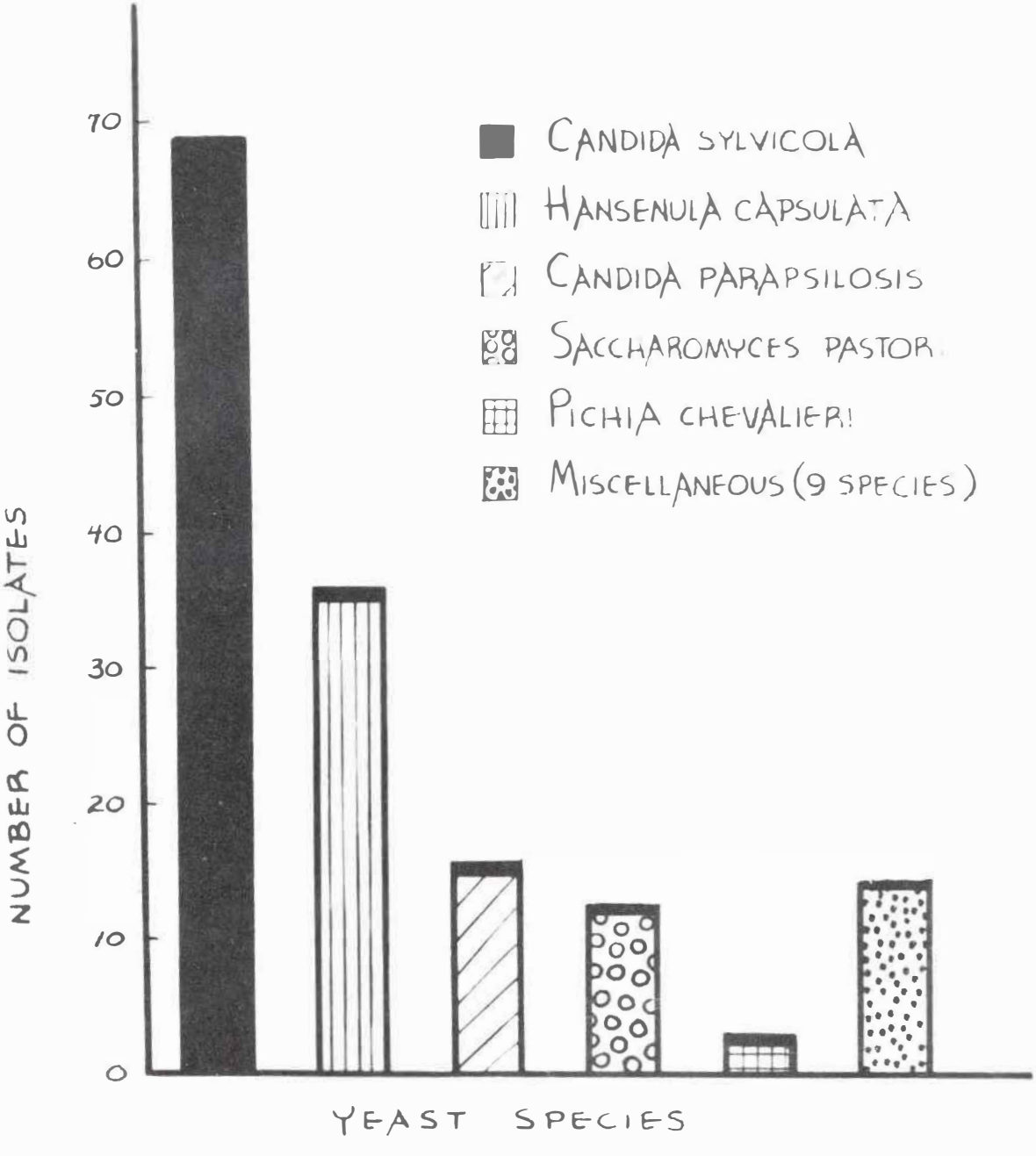
70
60
50
40
30
20
10
0
NUMBER OF ISOLATES
CANDIDA SYLVICOLA
HANSENULA CAPSULATA
CANDIDA PARAPSILOSIS
SACCHAROMYCES PASTOR
PICHIA CHEVALIERI
MISCELLANEOUS (9 SPECIES)
YEAST SPECIES

TABLE II

Yeasts isolated from Dendroctonus and Ips species

No. of isolates	Species of beetle	Meta-morphic stage	Taxonomic designation of isolates	Species of tree	Location of tree (county)
1	Dendroctonus jeffreyi	(A)[1]	Hansenula capsulata	Pinus jeffreyi	Lassen
2	" "	(A)	" "	" "	"
3	" "	(A)	" "	" "	"
4	" "	(A)	" "	" "	"
5	" "	(A)	" "	" "	"
6	" "	(A)	" "	" "	"
7	" "	(A)	Candida sylvicola	" "	"
8	" "	(A)	" "	" "	"
9	" "	(A)	" "	" "	"
10	" "	(A)	" "	" "	"
11	" "	(A)	Pichia haplophila	" "	"
12	" "	(A)	" "	" "	"
13	" "	(A)	Candida rugosa	" "	"
14	" "	(A)	Rhodotorula crocea	" "	"
15	" "	(A)	Moniliales	" "	"
16	" "	(A)	"	" "	"
17	Dendroctonus monticolae	(A)	Hansenula capsulata	Pinus ponderosa	"
18	" "	(A)	" "	" "	"
19	" "	(A)	" "	" "	"
20	" "	(A)	" "	" "	"
21	" "	(A)	" "	" "	"
22	" "	(A)	" "	" "	"
23	" "	(A)	" "	" "	"
24	" "	(A)	" "	" "	"
25	" "	(A)	" "	" "	"

TABLE II (continued)

No. of isolates	Species of beetle	Meta-morphic stage	Taxonomic designation of isolates	Species of tree	Location of tree (county)
26	Dendroctonus monticolae	(A)	Hansenula capsulata	Pinus ponderosa	Lassen
27	" "	(A)	" "	" "	Nevada[2]
28	" "	(CA)	" "	" "	Lassen
29	" "	(CA)	" "	" "	"
30	" "	(P)	" "	" "	"
31	" "	(P)	" "	" "	"
32	" "	(P)	" "	" "	"
33	" "	(P)	" "	" "	"
34	" "	(A)	" "	Pinus monticola	El Dorado
35	" "	(A)	" "	" "	"
36	" "	(CA)	" "	" "	"
37	" "	(CA)	" "	" "	"
38	" "	(CA)	" "	" "	"
39	" "	(CA)	" "	" "	"
40	" "	(P)	" "	" "	"
41	" "	(A)	" "	P. contorta var latifola	"
42	" "	(A)	" "	" " "	"
43	" "	(P)	" "	" " "	"
44	" "	(A)	Candida sylvicola	Pinus ponderosa	"
45	" "	(A)	" "	" "	"
46	" "	(CA)	" "	" "	"
47	" "	(CA)	" "	" "	"
48	" "	(P)	" "	" "	"
49	" "	(L)	" "	" "	"
50	" "	(L)	" "	" "	"
51	" "	(L)	" "	" "	"
52	" "	(P)	" "	Pinus monticola	El Dorado
53	" "	(A)	" "	P. contra var latifolia	Lassen

TABLE II (continued)

No. of isolates	Species of beetle	Meta-morphic stage	Taxonomic designation of isolates	Species of tree	Location of tree (county)
54	Dendroctonus monticolae	(A)	Candida sylvicola	P. contra var latifolia	Lassen
55	" "	(A)	" "	" "	"
56	" "	(A)	" "	" "	"
57	" "	(CA)	" "	Pinus monticola	El Dorado
58	" "	(P)	" "	" "	"
59	" "	(P)	" "	" "	"
60	" "	(P)	" "	" "	"
61	" "	(P)	" "	" "	"
62	" "	(L)	" "	" "	"
63	" "	(L)	" "	" "	"
64	" "	(L)	" "	" "	"
65	" "	(A)	Candida parapsilosis	Pinus ponderosa	Lassen
66	" "	(A)	" "	" "	"
67	" "	(A)	" "	" "	"
68	" "	(A)	" "	" "	"
69	" "	(A)	" "	" "	"
70	" "	(A)	" "	" "	"
71	" "	(A)	C. parapsilosis var lactosa	" "	"
72	" "	(A)	" "	" "	"
73	" "	(A)	" "	" "	Nevada
74	" "	(L)	" "	P. contra var latifolia	Lassen
75	" "	(F)	Saccharomyces pastori	Pinus monticola	El Dorado
76	" "	(A)	" "	" "	"
77	" "	(A)	" "	" "	"
78	" "	(A)	" "	" "	"
79	" "	(A)	" "	Pinus ponderosa	Nevada
80	" "	(CA)	" "	" "	Lassen
81	" "	(CA)	" "	Pinus monticola	El Dorado
82	" "	(L)	" "	" "	"
83	" "	(L)	Cryptococcus diffluens	Pinus ponderosa	"

TABLE II (continued)

No. of isolates	Species of beetle	Meta-morphic stage	Taxonomic designation of isolates	Species of tree	Location of tree (county)
84	Dendroctonus monticolae	(L)	Saccharomyces bisporus	Pinus ponderosa	Lassen
85	" "	(A)	Torulopsis nitratophila	" "	Nevada
86	" "	(A)	Torulopsis pinus	" "	El Dorado
87	" "	(P)	" "	" "	Lassen
88	" "	(A)	Candida mycoderma	Pinus monticola	El Dorado
89	" "	(F)	Hansenula sylvicola	" "	Lassen
90	Dendroctonus valens	(A)	Candida sylvicola	Pinus ponderosa	"
91	" "	(A)	" "	Pinus jeffreyi	"
92	Ips oregoni	(A)	Hansenula capsulata	Pinus ponderosa	"
93	"	(P)	" "	Pinus jeffreyi	"
94	"	(A)	Candida sylvicola	Pinus ponderosa	"
95	"	(A)	" "	" "	"
96	"	(A)	" "	" "	"
97	"	(L)	" "	" "	"
98	"	(A)	" "	Pinus jeffreyi	"
99	"	(A)	" "	" "	"
100	"	(A)	" "	" "	"
101	"	(A)	" "	" "	"
102	"	(CA)	" "	" "	"
103	"	(CA)	" "	" "	"
104	"	(CA)	" "	" "	"
105	"	(CA)	" "	" "	"
106	"	(CA)	" "	" "	"
107	"	(L)	" "	" "	"
108	"	(L)	" "	" "	"
109	"	(L)	" "	" "	"
110	"	(L)	" "	" "	"
111	"	(L)	" "	" "	"
112	"	(P)	" "	" "	"

TABLE II (continued)

No. of isolates	Species of beetle	Meta-morphic stage	Taxonomic designation of isolates	Species of tree	Location of tree (county)
113	Ips oregoni	(P)	Candida sylvicola	Pinus jeffreyi	Lassen
114	"	(P)	" "	" "	"
115	"	(P)	" "	" "	"
116	"	(A)	" "	P. contorta var latifolia	"
117	"	(A)	" "	" "	"
118	"	(A)	" "	" "	"
119	"	(A)	" "	" "	"
120	"	(A)	" "	" "	"
121	"	(A)	" "	" "	"
122	"	(A)	" "	" "	"
123	"	(A)	" "	" "	"
124	"	(A)	" "	" "	"
125	"	(A)	" "	" "	"
126	"	(L)	" "	" "	"
127	"	(A)	Candida parapsilosis	" "	"
128	"	(A)	Saccharomyces pastori	" "	"
129	"	(A)	Unidentified	" "	"
130	"	(L)	Candida mycoderma	Pinus ponderosa	"
131	Ips confusus	(A)	Hansenula capsulata	" "	El Dorado
132	"	(A)	Candida sylvicola	" "	"
133	"	(A)	" "	Pinus monticola	"
134	"	(A)	" "	Pinus lambertiana	"
135	"	(A)	Candida parapsilosis	Pinus ponderosa	"
136	"	(A)	C. parapsilosis var lactosa	" "	"
137	"	(A)	" "	" "	"
138	"	(A)	" "	" "	"
139	"	(A)	" "	" "	"
140	"	(A)	Saccharomyces pastori	" "	"

TABLE II (continued)

No. of isolates	Species of beetle	Meta-morphic stage	Taxonomic designation of isolates	Species of tree	Location of tree (county)
141	Ips confusus	(A)	Saccharomyces pastori	Pinus ponderosa	El Dorado
142	"	(A)	" "	Pinus monticola	"
143	"	(A)	" "	" "	"
144	"	(A)	Candida mycoderma	" "	"
145	Ips imarginata	(A)	Candida sylvicola	Pinus jeffreyi	Lassen
146	"	(A)	" "	" "	"
147	"	(A)	" "	" "	"
148	"	(A)	" "	" "	"
149	"	(A)	" "	" "	"
150	"	(A)	" "	" "	"
151	"	(A)	Pichia haplophila	" "	"

[1] (A) = Adults, (CA) = Callow-Adults, (P) = Pupae, (L) = Larvae, (F) = Adults in Flight.

[2] Crystal Bay, Lake Tahoe, Nevada.

metamorphic stages is shown in Table III. Forty-one yeasts were isolated from larvae, pupae, callow-adults and adults of D. monticolae collected from P. ponderosa. The number of yeasts isolated from each of the different stages in the life cycle of the beetle is shown in Table IV. Nineteen yeasts were isolated from the metamorphic stages of Ips oregoni, collected from P. jeffreyi. The number of isolates from each of the different stages in the life cycle is presented in Table V. The foregoing cases are the only ones in which the yeast flores for all metamorphic stages of a single beetle (excluding the eggs) were investigated. In most other cases only the adults were studied. In a few cases, e.g., D. valens from P. jeffreyi, only one yeast was isolated; such instances will not be discussed separately here. More isolations would be required before the significance of the yeasts found, could be evaluated.

All the isolates and their association with the different sources are recorded in Tables VI through IX.

2. Temperature tolerance of the yeasts

The activity of the yeast isolates at various temperatures was measured by growth. Six temperatures of incubation were used: 0°, 5°, 13°, 31°, 34°, and 39°C. The medium used for the test consisted of 1 per cent glucose and 0.5 per cent yeast extract in distilled water. The medium was put in 16 mm tubes and sterilized by autoclaving. The tubes received a light inoculum from young wort agar slant cultures. Reading of the results was done by Wickerham's method (1951). The results observed after two weeks are given in Table X. The ability of the miscellaneous yeasts to grow at the different temperatures is recorded in Table XI.

TABLE III

Yeasts isolated from the different metamorphic stages of Dendroctonus monticolae found in Pinus monticola

Stages in life cycle	Hansenula capsulata	Candida sylvicola	Candida parapsilosis	Saccharomyces pastori	Miscellaneous group	Total yield of yeasts
Larvae (5)[1]	0	3	0	1	0	4
Pupae (5)	1	4	0	0	0	5
Callow-Adults (6)	4	1	0	1	0	6
Adults before emergence (6)	2	0	0	3	1	6
Adults in flight (5)	0	1	0	1	1	3
Total (27)	7	9	0	6	2	24

1 = Number of individuals dissected.

TABLE IV

Yeasts isolated from the different metamorphic stages of Dendroctonus monticolae found in Pinus ponderosa

Stages in life cycle	Hansenula capsulata	Candida sylvicola	Candida parapsilosis	Saccharomyces pastori	Miscellaneous group	Total yield of yeasts
Larvae (5)[1]	0	3	0	0	2	5
Pupae (5)	3	1	0	0	1	5
Callow-Adults (5)	2	2	0	1	0	5
Adults before emergence (22)	12	2	9	1	2	26
Total (37)	17	8	9	2	5	41

1 = Number of individuals dissected.

TABLE V

Yeasts isolated from the different metamorphic stages of Ips oregoni found in Pinus jeffreyi

Stages in life cycle	Hansenula capsulata	Candida sylvicola	Candida parapsilosis	Saccharomyces pastori	Miscellaneous group	Total yield of yeasts
Larvae (5)[1]	0	5	0	0	0	5
Pupae (5)	1	4	0	0	0	5
Callow-Adults (5)	0	5	0	0	0	5
Adults before emergence (5)	0	4	0	0	0	4
Total (20)	1	18	0	0	0	19

1 = Number of individuals dissected.

TABLE VI

Yeasts isolated from *Ips* beetles[1]

SPECIES OF BEETLE	SPECIES OF PINE	SPECIES OF YEAST					
		H. capsulata	C. sylvicola	C.parapsilosis	S. pastori	Misc. Group	Total No. of Isolates
Ips confusus (5)[2]	Pinus monticola	0	1	0	2	1	4
Ips confusus (4)	Pinus lambertiana	0	1	0	0	0	1
Ips confusus (8)	Pinus ponderosa	1	1	5	2	0	9
Ips oregoni (9)	Pinus ponderosa	1	4	0	0	1	6
Ips oregoni (10)	Pinus contorta var latifolia	0	11	1	1	1	14
Ips oregoni (20)	Pinus jeffreyi	1	18	0	0	0	19
Ips imarginata (6)	Pinus jeffreyi	0	6	0	0	1	7
Total (62)		3	42	6	5	4	60

1 = Three *Ips confusus* beetles were dissected yielding no microbial growth. They were not included in this table.

2 = Number of individuals dissected.

TABLE VII

Yeasts isolated from Dendroctonus beetles[1]

SPECIES OF BEETLE	SPECIES OF PINE	SPECIES OF YEAST					
		H. capsulata	C. sylvicola	C.parapsilosis	S. pastori	Misc. Group	Total No. of Isolates
D. monticolae (37)[2]	P. monticola	7	9	0	6	2	24
D. monticolae (7)	P. contorta var latifolia	3	4	1	0	0	8
D. monticolae (27)	P. ponderosa	17	8	9	2	5	41
D. valens (2)	P. ponderosa	0	1	0	0	0	1
D. valens (1)	P. jeffreyi	0	1	0	0	0	1
D. jeffreyi (14)	P. jeffreyi	6	4	0	0	6	16
Total (88)		33	27	10	8	13	91

1 = Three Dendroctonus brevicomis beetles were dissected yielding no microbial growth. These beetles are not included in the table.

2 = Number of individuals dissected.

TABLE VIII

Yeasts isolated from recently attacked Pinus ponderosa and Pinus jeffreyi[1]

SPECIES OF BEETLE	SPECIES OF TREE	H. capsulata	C. sylvicola	C.parapsilosis	S. pasteri	Misc. Group	Total No. of Isolates
D. monticolae (20)[2]	Pinus ponderosa	8	7	0	1	4	20
Ips oregoni (20)	Pinus jeffreyi	1	18	0	0	0	19

1 = The yeasts reported in this table were isolated from beetles collected at Blacks Mountain Experimental Forest.

2 = Number of individuals dissected.

TABLE IX

Yeasts isolated from species of Dendroctonus and Ips attacking pine trees

	SPECIES OF YEAST											
	M. capsulata		C. sylvicola		C. parapsilosis		Sacch. pastori		Misc. Group		Total	
SPECIES OF TREE	D.[1]	Ips[2]	D.	Ips	D.	Ips	D.	Ips	D.	Ips	D.	Ips
Pinus jeffreyi	6	1	5	24	0	0	0	0	6	1	17	26
Pinus ponderosa	17	2	9	5	9	5	2	2	5	1	42	15
Pinus monticola	7	0	9	1	0	0	6	2	2	1	24	4
Pinus contorta var latifolia	3	0	4	11	1	1	0	1	0	1	8	14
Pinus lambertiana	0	0	0	1	0	0	0	0	0	0	0	1

1 = Yeasts reported in this column were isolated from species of Dendroctonus.

2 = Yeasts reported in this column were isolated from Ips species.

TABLE X

Temperature tolerance of yeasts

TEMPERATURE	ORGANISM							
	H. capsulata[1]		C. sylvicola[2]		C.parapsilosis[3]		S. pastori[4]	
	Growth		Growth		Growth		Growth	
	-[5]	+[6]	-	+	-	+	-	+
0°C.	15	2	29	6	6	8	12	0
5°C.	1	16	0	35	0	14	1	11
13°C.	0	17	0	35	0	14	0	12
31°C.	0	17	0	35	0	14	0	12
34°C.	1	16	5	30	0	14	8	4
39°C.	16	1	30	5	7	7	12	0

1 = Seventeen cultures of H. capsulata isolates were used in this experiment.

2 = Thirty-five cultures of the C. sylvicola isolates were used in this experiment.

3 = Fourteen cultures of C. parapsilosis isolates were used in this experiment.

4 = Twelve cultures of S. pastori isolates were used in this experiment.

5 = - represents poor to no growth.

6 = + represents moderate to good growth.

TABLE XI

Temperature tolerance of miscellaneous isolates

YEAST SPECIES	TEMPERATURE					
	0°C.	5°C.	13°C.	31°C.	34°C.	39°C.
Candida rugosa	-	+	+	+	-	-
Monilia sp.	-	-	-	+	+	-
Candida mycoderma[1]	-	-	-	+	+	-
T. pinus[2]	-	-	+	+	-	-
S. bisporus	-	+	+	+	+	+
Cryptococcus diffluens	-	+	+	+	-	-
Pichia haplophila[3]	-	-	+	+	-	-

1 = Three strains of C. mycoderma were tested in this experiment.

2 = Two strains of T. pinus were tested in this experiment.

3 = Three strains of P. haplophila were tested in this experiment.

It is evident, that all yeasts, with a few exceptions, belonging to the four major groups, can grow at temperatures ranging from 5° to 34°C. All yeasts, except the strains of C. parapsilosis and a few strains belonging to the other three main groups were unable to grow at either 0° or 39°C.

The ability of H. capsulata, C. sylvicola, C. parapsilosis, and S. pastori to grow at 5°C is significant because bark beetles are still inactive at this temperature. Thus, yeasts brought into the phloem of a pine, by a bark beetle, can grow and slowly ferment the phloem during cold weather. Consequently by the time that the beetles become active, the yeasts in the galleries may have started already to multiply and produce aromatic fermentative substances.

TAXONOMY OF THE YEASTS

Of the hundred and fifty-one isolates, fifty-four sporulated and ninety-seven were imperfects. The sporulating yeasts fell into the following genera: Hansenula, Saccharomyces and Pichia.

A. Genus Hansenula: Thirty-six isolates were found to be similar to H. capsulata Wickerham and one to H. sylvicola Wickerham.

Hansenula capsulata: Wickerham (1951) reported that strains of this species have been isolated from tunnels of larvae, and frass in Picea sp., Pinus divaricata, P. echinata, P. taeda, and P. ponderosa. Our isolates fit exactly the description of the species given by Wickerham, but for two instances when they failed to utilize alphamethylglucoside. This was tested both by the auxanographic technique and the replica

plating method. Rhamnose was utilized only by eighteen of the isolates. These compounds were reported by Wickerham to be assimilated sometimes weakly. These differences are therefore not sufficiently important to name these strains as varieties.

Hansenula sylvicola: This species also was described by Wickerham in 1951. He isolated three strains from gum of wild cherry, Prunus serotina. This species was reported to produce a sweet fruity odor, but no typical ester odor usually associated with the genus. The only isolate of this species is in accord with Wickerham's description.

B. Genus Saccharomyces: Thirteen strains of S. pastori and one of S. bisporus were isolated.

Saccharomyces pastori: In 1936 Holst described a new yeast Zygosaccharomyces pini, which he isolated regularly from Dendroctonus and other bark beetles or from Pinus species infested by them. Lodder and van Rij (1952) studied this species and found it was similar to S. pastori, except that the fermentation was very much weaker. They regarded it as a strain of S. pastori with reduced fermentation ability. Our isolates are all weak fermenters and resemble, in this respect, Holst's strain. Three of our isolates showed a weak galactose assimilation using both the auxanographic technique and the replica method. These might, therefore, be regarded as a transition group between S. pastori and Pichia haplophila.* Shihata (1951) isolated ten strains from Drosophila which he called S. pastori on the basis of Stelling-Dekker's description, which did not include the carbon assimilation pattern. Since nine of his isolates were able to assimilate glucose,

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*Cf. taxonomic discussion of P. haplophila nov. sp.

sucrose, and maltose, Shibata's strains are distinctly different from S. pastori, which can assimilate only glucose, according to Lodder and van Rij (1952). The main reason for including all these organisms with derby-hat shaped spores in Saccharomyces is the absence of a distinct film on malt extract. However, the author believes that they are closely related to Pichia and might be considered as strains of this genus in which the ability to form a pellicle has been lost. In order to define the bark beetle strains more accurately, the author tested the assimilation of raffinose, erythritol, rhamnose, alpha-methylglucoside and trehalose. These compounds were used by Wickerham for a more satisfactory delimitation of the species Hansenula. No correlation was found in the assimilation patterns of these compounds by the galactose positive group and galactose negative strains described above.

Saccharomyces bisporus. One strain of this species was studied by Beijerinck (Lodder and van Rij, 1952). It had been isolated from exudate of oak. Our strain is in accord with the description of the type species, with the exception that it contains one, two and three spores in the ascus, whereas the type strain has only one to two ascospores.

C. Genus Pichia.

Three strains belonging to this genus were isolated. Two of the strains were found to be identical and were isolated from the same beetle. The colonies on the original plate appeared somewhat distinct, explaining the isolations of the two strains. Because a distinct and dull film is formed on liquid wort as well as on alcohol medium and the spores are clearly hat shaped with a short brim, the organisms belong

in the genus Pichia. Because of the assimilation of both glucose and galactose, and the formation of hat shaped spores, these isolates cannot be identified with any of the known species of Pichia (Lodder and van Rij, 1952). It is therefore proposed to define them as new species. As the vegetative cells appear to occur entirely in the haploid condition the name of Pichia haplophila is chosen. One additional strain was isolated, which is identical in every way, except that the ability to utilize trehalose is lacking.

Standard description of Pichia haplophila nov. sp.

Growth in malt extract: After 24 hours in malt extract, cells round to oval, few elongate cells present (1.75-2.5)x(2.5-5) microns in size, single or in clusters. Complete thin dull pellicle, medium sediment. The streak culture after 3 weeks at room temperature, is white-cream, smooth, semi dull, soft, flat, with an entire border.

Slide culture: No pseudomycelium is formed.

Sporulation: Heterogamous conjugation precedes spore formation. The spores are small, hat shaped with short brims; 2-3 spores per ascus. Sporulation occurs readily on malt agar.

Fermentation:	Glucose -	Maltose -
	Galactose -	Lactose -
	Sucrose -	Raffinose -

Sugar assimilation:	Glucose +	Maltose -	Erythritol +
	Galactose +	Lactose -	Trehalose +
	Sucrose -	Raffinose -	

Assimilation of potassium nitrate: absent.

Ethanol as sole source of carbon: Complete, thin, white film, moderate growth.

Splitting of arbutin: absent.

There is a definite similarity between Pichia haplophila and three of the S. pastori isolates, in that both have hat shaped spores, both assimilate glucose and galactose, and neither ferment glucose (some of the S. pastori isolates can ferment glucose incompletely, but only after a prolonged incubation period). The one important character which separates the above two species is the ability of P. haplophila to form a pellicle both on malt extract and thanol.

It is generally accepted that spore shape is a more important character than pellicle formation. It therefore would appear that the pastori strains might fit better in the genus Pichia rather than in the genus Saccharomyces. This would require redefining the genus Pichia.

The nonsporulating yeasts belong to the following genera: Candida, Torulopsis, Rhodotorula, and Cryptococcus.

D. Genus Candida. Of the eighty-nine isolates belonging to this genus, 69 represent C. sylvicola nov. sp., 16 were similar to C. parapsilosis, 3 to C. mycoderma and 1 to C. rugosa.

Candida sylvicola nov. sp. This isolate is very similar to Hansenula sylvicola in its morphology, assimilation, and fermentation patterns, except that it does not produce spores. To preclude the possibility of nonsporulation because of heterothallism, a mixed suspension was made of 35 isolates. This was streaked on vegetable agar and carrot wedges (these were reported by Wickerham (1951) to be good sporulation media for H. sylvicola). Microscopic observations were made for two months without observation of conjugation or sporulation. Fifty-six strains can assimilate glucose, galactose, maltose, sucrose, trehalose,

alpha-methylglucoside, and nitrate. The other strains can assimilate all the above compounds with the exception of alpha-methylglucoside. Since no Candida species has been described which has these characteristics it was named Candida sylvicola nov. sp., because it is identical with Hansenula sylvicola but for the sporulation ability.

Strain 150 is designated as the type.

Standard description of Candida sylvicola nov. sp.

Growth in malt extract: After 24 hours at 25° C cells round to oval, (1.75-4)x(2-7) microns; mostly single or in pairs. After two weeks incubation, complete white film is formed and a moderate sediment.

Streak culture on malt agar: After three weeks at room temperature, cream-white, flat, semi-dull, soft, a little convex, with mycelial edge.

Slide culture: Pseudomycelium of long branched filament is formed. True mycelium, blastoconidia and blastospores are present.

Fermentation: Glucose +, Galactose +, Maltose -, Lactose -, Sucrose -

Sugar assimilation:* Glucose +, Galactose +, Maltose +, Sucrose +, Lactose -, Rhamnose +, Alpha-methylglucoside +, Trehalose +

Assimilation of potassium nitrate: Positive.

Ethanol as sole source of carbon: Moderate growth.

Splitting of arbutin: Positive.

The thirteen isolates that do not utilize alpha-methylglucoside will be regarded as strains of C. sylvicola which have lost the ability to

*The ability to assimilate trehalose, rhamnose, alpha-methylglucoside and erythritol, was determined, for all strains of C. sylvicola by the regular auxanographic technique and by the replica method.

assimilate this compound. No varietal designation is warranted for a strain with the inability to utilise the aforementioned compound.

Candida parapsilosis: This species is described by Lodder and van Rij (1952) as a pseudomycelium forming yeast, which assimilates glucose, galactose, sucrose, and maltose. Splitting of arbutin is negative. It ferments glucose, galactose (weakly), sucrose weakly or not at all, and maltose was weakly fermented by one strain only. No pellicle is formed in malt extract. C. parapsilosis var intermedia has the same characteristics except it has the ability to form a pellicle in malt extract, and is able to split arbutin. The arbutin test of our strains varied from distinctly positive, to weak and negative, whereas in all cases the film was poorly developed and consisted frequently of nothing but a few islets. This would indicate that the separation of the species C. parapsilosis and the variety intermedia is hard to maintain. However, the isolates form two distinct groups in accordance with their assimilation ability. One group, of 8 yeasts were found to assimilate lactose moderately, whereas the other eight cultures were negative when tested by auxanographic technique. As a result it was decided to place the later eight isolates in C. parapsilosis. However, it was felt, that the other eight isolates, with the ability to assimilate lactose, should be put in a new variety, to indicate the added ability of lactose utilization. The author proposes the name C. parapsilosis var. lactosa for these isolates.

Supplementary description of Candida parapsilosis var. lactosa.

Growth in malt extract: A very thin fragile pellicle may be formed. Some strains form only islets.

Slide culture: The pseudomycelium is moderately developed, both aerobically and anaerobically.

Sugar assimilation: Glucose - Lactose - (moderate)
Galactose - Maltose -
Sucrose -

Splitting of arbutin: Positive.

Candida mycoderma: Three cultures were isolated corresponding to C. mycoderma. They fit the description of the type species as given by Lodder and van Rij (1952). These yeasts have a very primitive pseudomycelium. Two of the isolates grow very well in ethanol, and rapidly form a complete dull pellicle, whereas the third isolate grows rather poorly in ethanol.

Candida rugosa: One yeast was placed in this species. It corresponds rather well to the standard description. It differs from the latter by having a very primitive pseudomycelium and by its inability to form a distinct pellicle in malt extract. These characteristics, however, do not warrant creation of a new variety.

E. Genus Torulopsis: Three isolates correspond to species in this genus.

Torulopsis pinus: This species was represented by two of the isolates, which checked well with the standard description, except for the ability of our strain to assimilate galactose moderately. Since the galactose assimilation is not strong and the organism otherwise corresponds very well to T. pinus formation of a new variety is not warranted. A third isolate could not be identified with any of the

described Torulopsis species. It is therefore considered as a new species for which the author proposes the name Torulopsis nitratophila, because of its good growth with nitrate as the only nitrogen source.

Standard description of Torulopsis nitratophila nov. sp.

Growth in malt extract: After 24 hours at 25°C cells are round to oval (1.75-3.5)x(2-5) microns, single or in pairs. Very thin incomplete film is formed, complete white ring and a little sediment, after two weeks incubation.

After three weeks at room temperature the streak culture is cream-brown, smooth, semi-dull, soft, flat, with an entire border.

Slide culture: No pseudomycelium is formed.

Fermentation: Absent.

Sugar assimilation: Glucose + Maltose -
Galactose + Lactose -
Sucrose -

Assimilation of potassium nitrate: Positive.

Ethanol as sole source of carbon: Poor growth.

Splitting of arbutin: Negative.

Starch formation: Negative.

F. Genus Rhodotorula.

Only one isolate was obtained belonging to this genus. Lodder and van Rij include in Rhodotorula those organisms possessing manifest red or yellow carotenoid pigments. This culture has a yellow color on wort agar and gives a very distinct yellow petroleum ether extract by the procedure of Mrak et al. (1949). Since it does not form a starch like compound under appropriate conditions, it definitely belongs to

Rhodotorula. Since it utilizes nitrate weakly and is lactose negative it does not fit in any of the described species. Because of its yellow color the name Rhodotorula crocea nov. sp. is proposed.

Standard description of Rhodotorula crocea nov. sp.

Growth in malt extract: After 48 hours at 25°C cells are oval to elongate, (1.75-3.5)x(3.5-9) microns, single or in pairs. After two weeks incubation incomplete thin film, wide yellow ring and medium sediment are formed.

Growth on malt agar: Ratio of length to width, in many cells, is 3 or 4 to 1.

The streak culture after three weeks at room temperature is dark yellow, smooth, glistening, soft to pasty, flat with an entire border.

Slide culture: No pseudomycelium is formed.

Fermentation: Absent.

Assimilation of sugars: Glucose +, Maltose +, Galactose +, Lactose -, Sucrose +

Assimilation of potassium nitrate: Positive.

Ethanol as sole source of carbon: No growth.

Splitting of arbutin: Weak.

Starch production under appropriate conditions is negative.

G. Genus Cryptococcus, one isolate.

Cryptococcus diffluens: One culture was isolated, which corresponds well to the description of the type species, although it does not form such a heavy slimy growth in old malt extract culture. This strain assimilates trehalose, alphamethylglucoside and rhamnose, but not raffinose and erythritol.

DISCUSSION

1. Association of yeasts and bark beetles. It is apparent from Table XXI that D. monticolae does not have any characteristic yeast flora. The twenty-four isolates are distributed quite evenly between H. capsulata, C. sylvicola, and S. pastori. However, C. sylvicola seems to be prevalent in the larval and pupal stages, and H. capsulata in the callow-adult and adult stages. Since the number of the isolates is quite small no definite conclusions, on the microfloral distribution in this beetle, can be made.

Table IV shows that the distribution of yeasts, in the different metamorphic stages of D. monticolae, collected from P. ponderosa, is variable. The increased number of H. capsulata among the isolates is the result of the larger number of adults dissected. No single yeast present which characterizes the whole group. However, the association of H. capsulata with the adult stages and its absence in the larval stage might indicate a change in the microflora with a change in the life cycle.

Eighteen out of 19 yeast isolated from I. oregoni are cultures of C. sylvicola. The adults of this species were collected at a different time than the larvae, pupae, and callow-adults. The latter were removed from three different pines; nevertheless, they all showed the dominance of C. sylvicola. This accentuates the fact that most of the yeasts in the microflora of I. oregoni beetle belong to a single species. Reasons for this association are not readily apparent.

Table VI tabulates all the yeasts isolated from Ips beetles. As can be seen from Table VI, 42 out of 60 yeasts (approximately 70%)

are C. sylvicola; therefore, this might justify the formulation of the hypothesis that the latter yeast is characteristically associated with beetles of this genus. That the different pines do not have a predominate yeast can be seen from Table IX, where all isolates are tabulated according to the tree in which they were found.

As was mentioned in the discussion on the collection of beetles, all of the first brood material had been infested in the fall of the year before collection. It had been in the field over winter. Consequently it might have been subject to attacks of secondary insects which could have caused a second yeast contamination of the phloem. It was impossible therefore to determine whether the yeasts found in the phloem of the infested pines were brought therein by the insects in question, or were there as a result of contamination. This is an important point because the microflora which was isolated might not be representative of the intestinal organisms of the beetles dissected. However, in the subsequent collection made at Blacks Mountain Experimental Forest the author ascertained that the pines from which he removed beetles had suffered only from attack of the insect in question. Yeasts isolated on this occasion are "authentic" yeasts. These isolates were used in compiling Tables IV, V and VIII.

From the data available at present we can conclude tentatively that neither the different species of pine nor species of Dendroctonus have a particular yeast flora. On the other hand the yeast flora associated with species of Ips seems to be distinct. A larger number of yeasts would have to be isolated before valid conclusions can be made.

2. Taxonomy of the yeasts isolated. One hundred and fifty-one yeasts were isolated in the study of the intestinal microflora of certain bark beetles belonging to the genus Dendroctonus and Ips.

The great majority of the isolates fall into four major groups: Candida sylvicola nov. sp. (69 cultures), Hansenula capsulata (36), Candida parapsilosis (15), and Saccharomyces pastori (13) which comprise 88% of the total. The miscellaneous yeasts isolated are: Pichia haplophila nov. sp. (3), Candida mycoderma (3), Saccharomyces bisporus (1), Candida rugosa (1), Torulopsis pinus (2), Torulopsis nitratophila nov. sp. (1), Hansenula sylvicola (1), Cryptococcus diffluens (1), Rhodotorula crocea nov. sp., two unidentified organisms apparently belonging to the family Moniliaceae, and one unidentified organism of uncertain taxonomic status. Thus it appears from these preliminary studies that the bark beetles investigated have a rather well defined microflora consisting of the above four groups. All the aforementioned strains, except those of C. parapsilosis, have been reported in the literature as present only in bark beetles or trees infested by them.

Of the total of 151 isolates, 111 cultures have the ability to assimilate nitrate as sole source of nitrogen. This is of considerable interest since in most yeast surveys reported in the literature, the number of nitrate positive yeasts is very small. The biological significance of this observation is not clear. Little is known about the nitrogen sources available to the yeast in the trees or in the intestinal tract of the beetles. Further studies would be desirable to elucidate this point. It should be borne in mind, however, that besides nitrate, these yeasts also are able to utilize ammonium ion or more complex nitrogen sources.

The thirteen isolates of S. pastori form hat-shaped spores, ferment glucose very slowly, but form no pellicle (or at the most film islets or an incomplete film). In the opinion of the writer the above-mentioned characters might warrant the removal of strains of pastori from the genus Saccharomyces and their inclusion in the genus Pichia. This would necessitate a revision in the generic definition of Pichia to allow inclusion of nonfilm-forming members. A similar situation exists in the genus Hansenula which originally contained only pellicle-forming species, but now also nonfilm formers. Ten of the S. pastori isolates could assimilate only glucose. The remaining three isolates could assimilate both glucose and galactose.

Three cultures of Pichia haplophila nov. sp., were isolated. These organisms resemble those strains of S. pastori which could assimilate galactose. The difference, however, was the ability of the strains of Pichia to form a pellicle on malt extract. This close similarity supports the suggestion that S. pastori should be placed in the genus Pichia. Furthermore by definition the genus Saccharomyces excludes all nonfermenting yeast.

Sixty-nine of the isolates were very similar to Hansenula sylvicola but for their inability to form ascospores. These yeasts were therefore named Candida sylvicola. Heterothallism apparently is not the reason for their inability to form ascospores, since in mixed cultures no spores were produced either (Leupold 1950, Wickerham 1950).

Fifteen cultures of C. parapsilosis were isolated. These yeasts varied from distinctly positive to weak and negative in their ability to split arbutin. Thus, the arbutin test seems to be inadequate for

the separation of C. parapsilosis and its variety intermedia as proposed by Lodder and van Rij. In all cases the film was poorly developed. Thus, some of the isolates which are arbutin positive, and have a poorly developed pellicle, fall in between C. parapsilosis and its variety intermedia. It seems, therefore, that there are arguments in favor of abolishing the variety intermedia and its inclusion in the species. Eight of these isolates were able to assimilate lactose. In view of this character a new variety (C. parapsilosis) var lactosa, was created.

Thirty-six cultures of Hansenula capsulata were isolated. These isolates fit the description of the species given by Wickerham but for the inability of two strains to utilise alphamethylglucoside.

Four new species, Candida sylvicola nov. sp., Pichia haplophila nov. sp., Rhodotorula crocea nov. sp., Torulopsis nitratophila nov. sp., and one variety Candida parapsilosis var lactosa were isolated and described.

SUMMARY AND CONCLUSIONS

1. Yeasts were isolated from Dendroctonus and Ips bark beetles collected from Pinus species in El Dorado and Lassen counties, California.
2. Some of the yeasts were isolated from the larval, pupal, callow-adult, and adult stages of Dendroctonus monticolae and Ips oregoni. The fact that yeasts were found in the nonfeeding metamorphic stages of the life cycle might indicate that the significance of these yeasts to the beetle is more than an accidental ingestion and digestion with other food components.
3. All yeasts were isolated by the use of a dissection procedure from the interior of the beetles, from one or more of the metamorphic stages, followed by streaking on wort-agar plates.
4. Morphological and physiological characters of the yeasts were determined by the procedures of the Dutch school, and those of Wickerham.
5. One hundred and fifty-one isolates were obtained. These isolates belong to seven genera, thirteen species, and one variety.
6. Fifty-four of the isolates were sporulating yeasts. This group is comprised of thirty-six isolates of Hansenula capsulata, thirteen Saccharomyces pastori, three Pichia haplophila nov. sp., one S. bisporus, and one H. sylvicola. Of the ninety-seven non-sporulating yeasts, sixty nine are termed Candida sylvicola nov. sp., eight C. parapsilosis, eight C. parapsilosis var lactosa nov. var., three C. mycoderma, one C. rugosa, two Torulopsis pinus, one T. nitratophila nov. sp., one Rhodotorula crocea nov. sp., one

Cryptococcus diffluens, two unidentified organisms apparently belonging to the family Moniliaceae, and one unidentified organism of uncertain taxonomic status.

7. Four new species, Candida sylvicola nov. sp., Pichia haplophila nov. sp., Rhodotorula crocea nov. sp., Torulopsis nitratophila nov. sp. and one variety (Candida parapsilosis) var. lactosa, were described.

8. Of the hundred and fifty-one isolates, four main groups emerge, C. sylvicola (69), H. capsulata (36), C. parapsilosis (16), and H. wingei (13), comprising 88% of the total.

9. Almost 74% of the yeasts isolated were able to grow with nitrate as the sole nitrogen source.

10. Seventy percent of the cultures isolated from Ips beetles were found to be C. sylvicola.

11. Isolates from Dendroctonus beetles seem to be fairly well distributed between the four major yeast groups indicated above.

12. Beetles were collected from Pinus jeffreyi, ponderosa, monticola, contorta var latifolia, and lambertiana. Insufficient data are available at present to state with certainty that any of the above pines have selective effect on the yeast flora present in the beetles obtained from them.

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Western Pine Beetle Investigations 1925-1930

No. 14. The Concentration of Certain Sugars in the Bark of the Western Yellow Pine, As related to Western Pine Beetle Attraction.

AGRICULTURAL RESEARCH SERVICE
Forest Insect Laboratory
29 Forestry Bldg., U. C.
Berkeley 4, California

December 17, 1953

To: Dr. J. A. Beal, in Charge, Forest Insect Investigations
USDA, BEPQ, Washington 25, D. C.

From: C. B. Kaston, in Charge, Forest Insect Laboratory

Subject: Thesis: The association of yeasts with certain bark beetles

Enclosed is a copy of subject Master's thesis by Moshe Shifrine. Shifrine is an Israeli graduate student at the University of California who undertook this work for us on a contract basis during the summer of 1952, under the immediate supervision of Mr. Callahan. He subsequently completed the studies for his Master's degree. We believe this work illustrates an excellent means of obtaining information of a very technical, non-entomological nature.

The thesis itself is not complete in that all of the California bark beetles, with which we are concerned, were not included; however, this situation has been rectified this past summer. A follow-up report will be prepared on this phase of the work. This dissertation is a unique one in that it is the first such survey of a large number of bark beetle species with respect to their yeast flora. The difference found in the yeast floras of Ips species versus Dendroctonus species is very interesting and may be worthy of further study.

As you know, we attempted to study the attractivity of these yeasts and their ferments during the past summer. Although these efforts were not successful, we consider that further work along these lines would be worthwhile. Now that we know with which microorganisms we are concerned, it is time to investigate the significance of the yeast - bark beetle relationship.

Only a limited number of copies of this thesis have been prepared, and these will be distributed as shown below.

cc: Portland, Coeur d'Alene, Fort Collins, Gulfport, Asheville, Albuquerque, Ogden, New Haven.